10320 Camino Santa Fe, Suite G
San Diego, CA 92121
Tel: 858.875.1900 Fax: 858.875.1999

Anti-RAGE/AGER Antibody Picoband™ (monoclonal, 5C6C1)

Catalog # ABO16627

Specification

Anti-RAGE/AGER Antibody Picoband™ (monoclonal, 5C6C1) - Product Information

Application	**WB, IHC, FC**
Primary Accession	Q15109
Host	**Mouse**
Isotype	**IgG2b**
Reactivity	**Rat, Human, Mouse**
Clonality	**Monoclonal**
Format	**Lyophilized**

Description
Anti-RAGE/AGER Antibody Picoband™ (monoclonal, 5C6C1) . Tested in Flow Cytometry, IHC, WB applications. This antibody reacts with Human, Mouse, Rat.

Reconstitution
Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml.

Anti-RAGE/AGER Antibody Picoband™ (monoclonal, 5C6C1) - Additional Information

Gene ID 177

Other Names
Advanced glycosylation end product-specific receptor, Receptor for advanced glycosylation end products, AGER, RAGE

Calculated MW
43 kDa KDa

Application Details
Western blot, 0.25-0.5 µg/ml, Mouse, Rat
 Immunohistochemistry(Paraffin-embedded Section), 2-5 µg/ml, Mouse, Rat
 Flow Cytometry, 1-3 µg/1x10^6 cells, Human

Contents
Each vial contains 4 mg Trehalose, 0.9 mg NaCl and 0.2 mg Na2HPO4.

Immunogen
A synthetic peptide corresponding to a sequence at the N-terminus of human RAGE, different from the related mouse and rat sequences by six amino acids.

Purification
Immunogen affinity purified.

Storage	**At -20°C for one year from date of receipt. After reconstitution, at 4°C for one month. It can also be aliquotted and stored frozen at -20°C for six months. Avoid repeated freezing and thawing.**

Anti-RAGE/AGER Antibody Picoband™ (monoclonal, 5C6C1) - Protein Information

Name AGER

Synonyms RAGE

Function
Cell surface pattern recognition receptor that senses endogenous stress signals with a broad ligand repertoire including advanced glycation end products, S100 proteins, high-mobility group box 1 protein/HMGB1, amyloid beta/APP oligomers, nucleic acids, phospholipids and glycosaminoglycans (PubMed:27572515, PubMed:28515150, PubMed:34743181). Advanced glycosylation end products are nonenzymatically glycosylated proteins which accumulate in vascular tissue in aging and at an accelerated rate in diabetes (PubMed:21565706). These ligands accumulate at inflammatory sites during the pathogenesis of various diseases, including diabetes, vascular complications, neurodegenerative disorders, and cancers and RAGE transduces their binding into pro-inflammatory responses. Upon ligand binding, uses TIRAP and MYD88 as adapters to transduce the signal ultimately leading to the induction or inflammatory cytokines IL6, IL8 and TNFalpha through activation of NF-kappa-B (PubMed:21829704, PubMed:33436632). Interaction with S100A12 on endothelium, mononuclear phagocytes, and lymphocytes triggers cellular activation, with generation of key pro-inflammatory mediators (PubMed:19386136). Interaction with S100B after myocardial infarction may play a role in myocyte apoptosis by activating ERK1/2 and p53/TP53 signaling (By similarity). Contributes to the translocation of amyloid- beta peptide (ABPP) across the cell membrane from the extracellular to the intracellular space in cortical neurons (PubMed:19906677). ABPP- initiated RAGE signaling, especially stimulation of p38 mitogen- activated protein kinase (MAPK), has the capacity to drive a transport system delivering ABPP as a complex with RAGE to the intraneuronal space. Participates in endothelial albumin transcytosis together with HMGB1 through the RAGE/SRC/Caveolin-1 pathway, leading to endothelial hyperpermeability (PubMed:27572515). Mediates the loading of HMGB1 in extracellular vesicles (EVs) that shuttle HMGB1 to hepatocytes by transferrin-mediated endocytosis and subsequently promote hepatocyte pyroptosis by activating the NLRP3 inflammasome (PubMed:34743181). Promotes also extracellular hypomethylated DNA (CpG DNA) uptake by cells via the endosomal route to activate inflammatory responses (PubMed:24081950, PubMed:28515150).

Cellular Location
[Isoform 1]: Cell membrane; Single-pass type I membrane protein [Isoform 10]: Cell membrane; Single-pass type I membrane protein

Tissue Location
Endothelial cells.

Anti-RAGE/AGER Antibody Picoband™ (monoclonal, 5C6C1) - Protocols

10320 Camino Santa Fe, Suite G
San Diego, CA 92121
Tel: 858.875.1900 Fax: 858.875.1999

Provided below are standard protocols that you may find useful for product applications.

- Western Blot
- Blocking Peptides
- Dot Blot
- Immunohistochemistry
- Immunofluorescence
- Immunoprecipitation
- Flow Cytomety
- Cell Culture

Anti-RAGE/AGER Antibody Picoband™ (monoclonal, 5C6C1) - Images

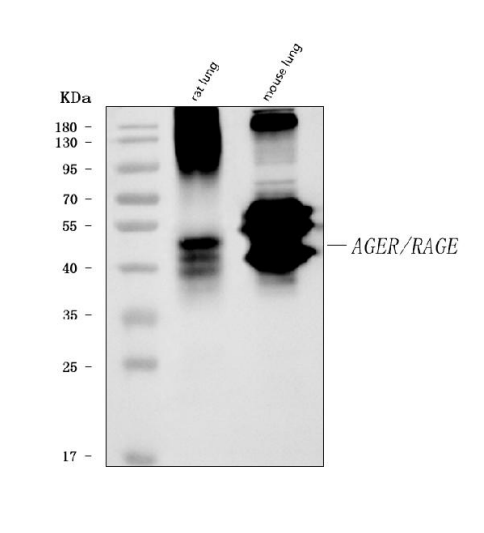

Figure 1. Western blot analysis of RAGE/AGER using anti-RAGE/AGER antibody (M03438-2). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.
Lane 1: rat lung tissue lysates,
Lane 2: mouse lung tissue lysates.
After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-RAGE/AGER antigen affinity purified monoclonal antibody (Catalog # M03438-2) at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for RAGE/AGER at approximately 43 kDa. The expected band size for RAGE/AGER is at 43 kDa.

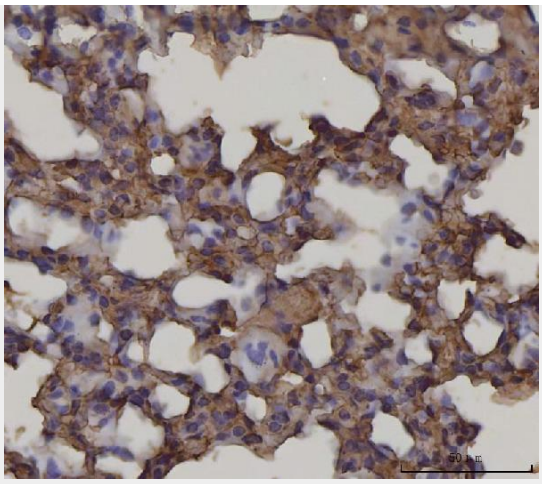

Figure 2. IHC analysis of RAGE/AGER using anti-RAGE/AGER antibody (M03438-2).
RAGE/AGER was detected in a paraffin-embedded section of mouse lung tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-RAGE/AGER Antibody (M03438-2) overnight at 4°C. Peroxidase Conjugated Goat Anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Mouse IgG Super Vision Assay Kit (Catalog # SV0001) with DAB as the chromogen.

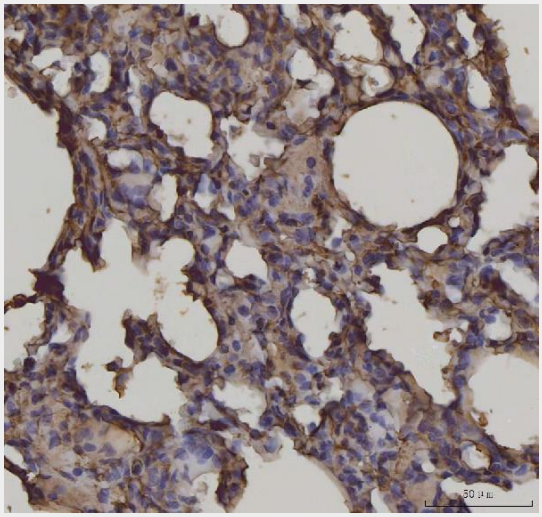

Figure 3. IHC analysis of RAGE/AGER using anti-RAGE/AGER antibody (M03438-2).
RAGE/AGER was detected in a paraffin-embedded section of rat lung tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-RAGE/AGER Antibody (M03438-2) overnight at 4°C. Peroxidase Conjugated Goat Anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Mouse IgG Super Vision Assay Kit (Catalog # SV0001) with DAB as the chromogen.

10320 Camino Santa Fe, Suite G
San Diego, CA 92121
Tel: 858.875.1900 Fax: 858.875.1999

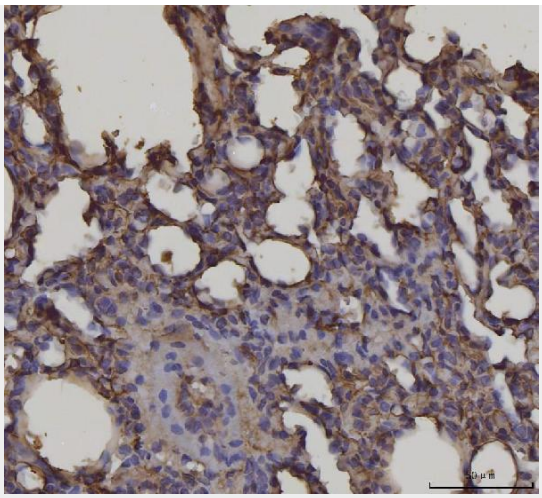

Figure 4. IHC analysis of RAGE/AGER using anti-RAGE/AGER antibody (M03438-2).
RAGE/AGER was detected in a paraffin-embedded section of rat lung tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-RAGE/AGER Antibody (M03438-2) overnight at 4°C. Peroxidase Conjugated Goat Anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Mouse IgG Super Vision Assay Kit (Catalog # SV0001) with DAB as the chromogen.

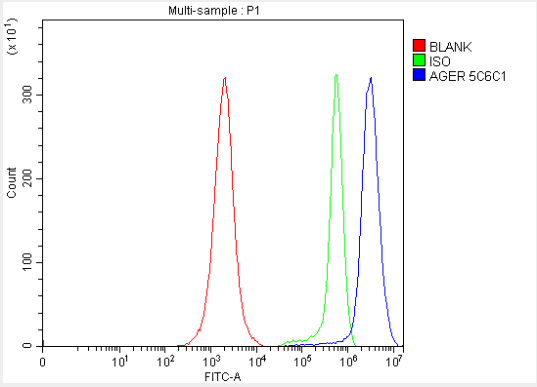

Figure 5. Flow Cytometry analysis of Jurkat cells using anti-RAGE/AGER antibody (M03438-2).
Overlay histogram showing Jurkat cells stained with M03438-2 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-RAGE/AGER Antibody (M03438-2, 1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

Anti-RAGE/AGER Antibody Picoband™ (monoclonal, 5C6C1) - Background

The receptor for advanced glycation end products (RAGE) is a multi-ligand member of the immunoglobulin superfamily of cell surface molecules. It interacts with distinct molecules implicated in homeostasis, development and inflammation, and certain diseases such as diabetes and Alzheimer's disease. RAGE is also a central cell surface receptor for amphoterin and EN-RAGE. And RAGE is associated with sustained NF-kappaB activation in the diabetic microenvironment and has a central role in sensory neuronal dysfunction. Moreover, RAGE propagates cellular dysfunction in several inflammatory disorders and diabetes, and it also functions as an endothelial adhesion receptor promoting leukocyte recruitment.